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Vaccines protect chickens against H5 highly pathogenic avian influenza in the face of genetic changes in field viruses over multiple years

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Abstract

Inactivated whole avian influenza (AI) virus vaccines, baculovirus-derived AI haemagglutinin vaccine and recombinant fowlpoxvirus-AI haemagglutinin vaccine were tested for the ability to protect chickens against multiple highly pathogenic (HP) H5 AI viruses. The vaccine and challenge viruses, or their haemagglutinin protein components, were obtained from field AI viruses of diverse backgrounds and included strains obtained from four continents, six host species, and isolated over a 38-year-period. The vaccines protected against clinical signs and death, and reduced the number of chickens shedding virus and the titre of the virus shed following a HP H5 AI virus challenge. Immunization with these vaccines should decrease AI virus shedding from the respiratory and digestive tracts of AI virus exposed chickens and reduce bird-to-bird transmission. Although most consistent reduction in respiratory shedding was afforded when vaccine was more similar to the challenge virus, the genetic drift of avian influenza virus did not interfere with general protection as has been reported for human influenza viruses.

Keywords: Avian influenza; Chickens; Immunity; Influenza; Vaccine

1. Introduction

Avian influenza (AI) occurs worldwide and is caused by Type A orthomyxoviruses of 15 haemagglutinin (H1–H15) and nine neuraminidase subtypes (Easterday et al., 1997).

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AI viruses can infect a diverse variety of bird species, including domestic poultry. With AI virus, there are two main pathotypes based upon infections of chickens and turkeys: (1) mildly pathogenic (MP) AI typically associated with respiratory disease, drops in egg production and/or mild to moderate increases in mortality and (2) highly pathogenic (HP) AI typically producing severe systemic disease, and high losses (Swayne et al., 1998; Perdue et al., 2000). In developed countries, AI viruses are not endemic in integrated commercial poultry systems, and control measures are taken to prevent introduction of AI or to eliminate AI if sporadic outbreaks occur of MP or HP AI.

Historically, individual nations have chosen control or eradication strategies to meet domestic needs and, to a lesser extent, requirements for international commerce of poultry and poultry products (Lancaster, 1981). Such strategies vary from tolerance of infections in poultry to a multi-faceted control program utilizing education of farmers and workers on avian influenza control, enhanced biosecurity on farms, increased surveillance and diagnostics, implementation of quarantine zones and controlled movement of birds within infected areas, and some method of eliminating infected birds (Lancaster, 1981). In addition, vaccines have been used in the US since the late 1970s for control of sporadic outbreaks of MP AI in turkeys (Lancaster, 1981). Recently, vaccines have been used in control programs for HP AI outbreaks in chickens in Mexico and Pakistan (Naeem and Hussain, 1995). In USA, the Animal and Plant Health Inspection Service has adopted a strategy for future control of HP AI that could potentially include vaccine use in emergency eradication program (Myers and Morgan, 1998).

By contrast, influenza is endemic in the human population and yearly vaccination is practised commonly throughout the developed nations (Murphy and Webster, 1996). An inactivated trivalent vaccine is central to the control and prevention of types A and B influenza, but because of antigenic drift of AI viruses in the field, the vaccines have a finite life span and the strain composition of the vaccine is changed on an annual basis (CDC, 1998).

The following studies were conducted to determine if H5 AI vaccines would need to be changed on a frequent basis in order to overcome antigenic changes in the field over time and maintain efficacy in chickens.

2. Materials and methods

2.1. Experiment 1: inactivated avian influenza virus vaccines

Groups of 10 4-week-old specific-pathogen-free (SPF) White Leghorn (WL) chickens obtained from flocks maintained at Southeast Poultry Research Laboratory were immunized subcutaneously with each of the 12 candidate inactivated vaccines prepared as described (Stone, 1987). Vaccines used included an uninoculated egg fluid (sham), a H7 AI virus and 10 H5 AI viruses (Table 1). Challenge was at 3 weeks post-vaccination (PV) by intranasal-(IN)-inoculation of $10^{7.7}$ mean embryo lethal doses (ELD_{50}) of HP A/chicken/Queretaro/14588/95 (H5N2) (Q1/95) AI virus. Oropharyngeal and cloacal swabs were taken at the peak of virus shedding, Day 3 post-inoculation (PI), for virus isolation attempts in 10 day embryonating chicken eggs using methods previously described

Table 1
Haemagglutinin (HA) protein similarity, clinical signs and death rates for chickens immunized at 4 weeks of age with inactivated avian influenza vaccines and challenged intranasally 3 weeks later with highly pathogenic A/Chicken/Queretaro/14588/95 (H5N2) virus

Vaccine virus	Abbreviations	HA protein similarity with challenge virus (%)	No. of chickens with clinical signs/total	No. of deaths/total
Sham	Sham	0	10/10	9/10
A/Turkey/Oregon/71 (H7N3)	TO/71	35.9	10/10	9/10
A/Turkey/Wisconsin/68 (H5N9)	TW/68	91.9	1/10	1/10
A/Mallard/Ohio/556/87 (H5N9)	MO/87	93.1	0/10	0/10
A/Chicken/Mexico/31381-7/94 (H5N2)	M10/93	96.9	0/10	0/10
A/Chicken/Mexico/26654-1374/94 (H5N2)	M5/94	95.4	1/10	1/10
A/Turkey/Minnesota/10734-5/95 (H5N2)	TM/95	92.5	0/10	0/10
A/Chicken/Jalisco/14589-660/94 (H5N2)	J12/94	97.9	0/10	0/10
A/Chicken/Queretaro/14588-19/95 (H5N2)	Q1/95	100	0/10	0/10
A/Chicken/Veracruz/28159-398/95 (H5N2)	V1/95	97.9	1/10	1/10
A/Chicken/Puebla/28159-474/95 (H5N2)	P3/95	93.1	1/10	1/10
A/Chicken/Chiapas/28159-488/95 (H5N2)	C4/95	96.7	0/10	0/10

(Swayne et al., 1998). The HA1 putative amino acid sequences were obtained from GenBank and similarity for vaccine and challenge viruses were determined as previously described (Swayne et al., 1999).

Spearman rank correlation (r_s) was used to test association between sequence homology of vaccine and challenge virus haemagglutinin, and reductions in titres of challenge virus shed from cloaca and oropharynx. Spearman rank correlations were performed on PC-based software (SigmaStat, Jandel Scientific, San Rafael, CA).

2.2. Experiment 2: baculovirus-derived influenza haemagglutinin protein vaccine

Ten 1-day-old White Plymouth Rock chickens were immunized with a baculovirus-derived vaccine containing a haemagglutinin gene insert from A/chicken/Jalisco/14589-660/94 (H5N2). Each chicken received 250 haemagglutinating units of protein in water-in-mineral-oil adjuvant given subcutaneously in 0.2 ml (Crawford et al., 1999). At 4 weeks PV, five chickens were challenged intranasally with 10^4 mean chicken lethal doses (CLD_{50}) of Q1/95 AI virus and five with A/chicken/Pennsylvania/1370/83 (H5N2) (CP/83) AI virus. Similar samples and analyses were taken as in Section 2.1 except challenge virus re-isolates were not titrated.

2.3. Experiment 3: recombinant fowlpoxvirus vaccine containing an influenza gene insert

Ninety 1-day-old SPF WL chickens were immunized with a recombinant fowlpox vectored vaccine containing a haemagglutinin gene insert from A/turkey/Ireland/83 (H5N8) (fowlpox-HA) (Swayne et al., 1997, 2000). Ninety chickens were also immunized with the fowl pox vector (fowlpox-Control). At 3 weeks PV, 10 fowlpox-

HA and 10 fowlpox-Control immunized chickens were IN challenged with $10^{2.4}$ CLD_{50} of one of nine different HP AI viruses (Table 3). Similar samples were taken as in Section 2.1.

3. Results

3.1. Experiment 1: inactivated avian influenza virus vaccines

The HA1 segment of the haemagglutinin protein from the H5 vaccine viruses had 91.9–100% deduced amino acid sequence similarity to the HA1 of the HP Q1/95 H5 challenge virus. The heterologous H7 AI vaccine had 35.9% similarity with the HA1 of the H5 challenge AI virus. The H5 vaccine and challenge AI viruses were of the North American lineage of H5 AI viruses.

For the H5 vaccine groups, most vaccinated chickens lacked clinical signs and survived challenge by a H5 HP Q1/95 AI virus (Table 1) while most chickens in the sham and H7 vaccine groups had clinical signs (100%) and died (90%) following the same challenge. The AI challenge virus was recovered frequently (83%) from the oropharynx of chickens from all 12 groups on Day 3 PI. The titres of AI virus recovered were $10^{1.3}$–$10^{3.5}$ ELD_{50}/ml of swab media lower for H5 vaccinates as compared to the sham or H7 vaccine groups. AI virus was recovered less frequently from the cloaca of H5 vaccinated chickens (<20%) when compared to the sham and H7 groups (60%). The virus titres were low for all cloacal samples from sham, H7 and H5 vaccine groups. There was no correlation between deduced amino acid sequence similarity of the vaccines and challenge virus, and the ability to reduce the quantity of challenge virus isolated from the oropharynx (P=0.10) and cloaca (P=0.97) on Day 3 PI.

3.2. Experiment 2: baculovirus-derived influenza haemagglutinin protein vaccine

The haemagglutinin protein of the vaccine had 87.6 and 97.6% similarity to haemagglutinin proteins of Q1/95 and CP/83 challenge AI viruses, respectively (Table 2). The vaccine prevented deaths following lethal challenge by Q1/95 and CP/83 (Table 2).

Table 2
Haemagglutinin (HA) protein similarity, deaths and challenge virus recovery rates for chickens immunized with the baculovirus-derived H5 haemagglutinin protein vaccine at 1 day of age and challenged 3 weeks later with highly pathogenic A/chicken/Queretaro/14588/95 (H5N2) virus

Challenge virus	HA protein similarity with challenge virus (%)	Deaths	No. of chickens with challenge virus recovery/total	
			Oropharynx	Cloaca
A/chicken/Queretaro/14588-19/95 (H5N2) (Q1/95)	97.6	0/5	5/5	0/5
A/chicken/Pennsylvania/1370/83 (H5N2) (CP/83)	87.6	0/5	5/5	0/5

Table 3
Haemagglutinin (HA) protein similarities and death rates for chickens immunized with fowlpox-Control (Control) and fowlpox-HA (HA) vaccines at 1 day of age and challenged 3 weeks later with nine different H5 highly pathogenic AI viruses

Challenge avian influenza virus	Abbreviations	HA protein similarity with vaccine (%)	Number of deaths/total	
			Fowlpox-control	Fowlpox-HA
A/turkey/Ireland/83 (H5N8)	TI/83	100	10/10	0/10
A/turkey/England/91 (H5N1)	TE/91	94.2	10/10	0/10
A/tern/South Africa/61 (H5N3)	TSA/61	93.1	10/10	0/10
A/chicken/Scotland/59 (H5N1)	CS/59	92.0	9/10	0/10
A/human/Hong Kong/156/97 (H5N1)	HK/97	90.2	8/10	0/10
A/chicken/Queretaro/14588-19/95 (H5N2)	Q1/95	89.3	1/10	0/10
A/turkey/Ontario/77322/66 (H5N9)	TO/66	89.1	9/10	0/10
A/emu/TX/399924/93 (H5N2)[a]	ET/93	88.8	7/10	0/10
A/chicken/Pennsylvania/1370/83 (H5N2)	CP/83	87.3	10/10	0/10

[a] HP virus was derived by laboratory passage in 14-day embryonating chicken eggs (Swayne et al., 1996).

Challenge virus was recovered from oropharynx but not the cloaca of all chickens on Day 3 PI (Table 2).

3.3. Experiment 3: recombinant fowlpoxvirus vaccine containing an influenza gene insert

The haemagglutinin protein had 87.3–100% deduced amino acid sequence similarity between the nine H5 challenge AI viruses and the Fowlpox-HA vaccine virus (Table 3). The vaccine contained a haemagglutinin gene insert from a Eurasian lineage H5 AI virus and had 90.2–100% haemagglutinin deduced amino acid sequence similarity with the five Eurasian lineage H5 challenge viruses (Table 3). Furthermore, the vaccine had 87.3–89.3% haemagglutinin deduced amino acid sequence similarity with the four North American lineage H5 challenge viruses (Table 3).

The Fowlpox-HA vaccinated chickens had no deaths following lethal challenge with the nine H5 HP AI viruses while 70–100% of Fowlpox-control vaccinated chickens died after the same AI virus challenge (Table 3). Commonly, AI virus was recovered from the oropharynx (90%) and cloaca (88%) of Fowlpox-Control vaccinated chickens that were challenged with HP AI viruses and sampled on Day 3 PI. By contrast, chickens in the Fowlpox-HA groups had less frequent recover of the challenge virus from the oropharynx (27%) and cloaca (3%) than in corresponding Fowlpox-Control groups. In most Fowlpox-HA groups, <20% of the chickens had AI virus recovered from the oropharynx and cloaca on Day 3 PI, except CP/83 and Q1/95 Fowlpox-HA groups had 100 and 90% isolation rates from the oropharynx, respectively.

Vaccination with Fowlpox-HA significantly reduced the titres of challenge AI virus recovered from the oropharynx (10^2–10^4 ELD_{50}/ml) and cloaca (10^1–10^3 ELD_{50}/ml) in all groups, except for oropharynx from C1/95 and CP/83 groups. However, there was no correlation between haemagglutinin sequence similarity of vaccine and challenge viruses, and reduction in virus titres shed from the cloaca (P=0.78), but there was a direct

correlation between the sequence similarity of the haemagglutinin and the ability to reduce virus titres shed from the oropharynx (P=0.009).

4. Discussion

The inactivated whole AI virus vaccines, baculovirus-derived AI haemagglutinin vaccine and recombinant fowlpoxvirus-AI haemagglutinin vaccine protected chickens from clinical signs and death following lethal challenge by multiple HP H5 AI viruses. The vaccine and challenge viruses, or their haemagglutinin protein components, were from field AI viruses of diverse backgrounds and included strains obtained from four different continents (North America, Europe, Asia and Africa), six different host species (chicken, turkey, mallard, emu, tern and human) and isolated over a 38-year-period (1959–1997). Furthermore, vaccination against AI reduced the number of chickens from which AI challenge virus could be re-isolated from the cloaca and oropharynx, and decreased the titres of virus detected in the cloaca and oropharynx which suggests that vaccination has the potential to reduce environmental contamination with AI virus and prevent subsequent bird-to-bird transmission. Previously, the recombinant fowlpoxvirus vaccine was shown to reduce contact transmission of a HP H5 AI virus from Mexico (Swayne et al., 1997).

Several issues that impact avian influenza vaccine efficacy were addressed. First, in Experiment 1, protection against a HP H5 AI challenge virus was demonstrated using ten different North American lineage H5 vaccine virus strains having 89–100% haemagglutinin protein sequence similarity with the challenge virus. This protection was against clinical signs and death, and in reducing the quantity of challenge virus shed from vaccinated chickens as compared to sham vaccinates. However, there was no statistical correlation between reduction in titres of virus shed from the oropharynx and cloaca, and haemagglutinin sequence similarity between the vaccine and challenge viruses.

Second, in Experiment 2 using a baculovirus-derived subunit vaccine, the haemagglutinin protein alone was shown to provide protection against clinical signs and death following challenge by two HP H5 AI viruses with 87.6 and 97.6% haemagglutinin protein sequence similarity to the vaccine. In addition, no challenge AI virus was isolated from the cloaca of vaccination chickens, but all chickens had virus re-isolated from the oropharynx suggesting vaccine use may reduce, but will not totally prevent field virus replication.

Third, in Experiment 3 with a recombinant fowlpoxvirus vaccine, the AI haemagglutinin in the vaccine provided broad-based protection against clinical signs and death following challenge by nine different HP H5 AI viruses. The challenge viruses represented both North American and Eurasian lineages of AI viruses, and had 87.3–100% haemagglutinin protein sequence similarity with vaccine. The vaccine also reduced the number of chickens from which AI virus was recovered from the cloaca and oropharynx and reduced the titers of the recovered virus. There was an association between the haemagglutinin protein sequence homology and the ability to reduce shedding from the oropharynx, but not from the cloaca. This association correlated statistically with the haemagglutinin sequence similarity of vaccine and challenge virus,

but more importantly, the reduction in oropharyngeal viral titers was most consistent for challenge viruses within the same lineage as the vaccine; i.e. Eurasian lineage of AI viruses.

This broad-based protection against HP AI isolates in poultry over multiple years is in contrast to human influenza where vaccines are changed yearly to provide optimal protection in the face of antigenic drift in seasonal field viruses (CDC, 1998). Some of the disparity may be accounted by the differences in pathogenesis of the disease caused by influenza virus in poultry versus humans. In chickens and turkeys, the highly pathogenic AI viruses caused severe systemic disease with replication of virus in multiple visceral organs, the brain and blood vascular endothelial cells (CDC, 1998). Serum antibodies elicited by haemagglutin in subtype specific vaccines or field virus exposure have been protective in preventing clinical signs and death (CDC, 1998). However, prevention of virus replication in and shedding from the respiratory and digestive tracts may require optimization of specific humoral immunity (Ruben, 1987). By contrast, influenza is predominantly an upper respiratory disease of humans with some high risk groups developing severe disease including interstitial pneumonia, neurological disease and other complications (CDC, 1998). Vaccines have been shown to prevent the manifestations of disease by about 30–70% in all populations, and have reduced deaths in high-risk individuals by about 60–87% (Ruben, 1987).

From a pragmatic view, our results show that the current H5 AI vaccines can provide protection against a variety of HP H5 AI viruses and frequent changing of the AI virus strain or the haemagglutinin portion of the recombinant or subunit vaccines may not be necessary to provide protection against HP AI viruses in the field. Immunization with these vaccines should decrease AI virus shedding from the respiratory and digestive tracts of AI virus exposed chickens. However, viruses with <90% homology at the haemagglutinin protein between vaccine and challenge virus may not result in consistent reductions in AI challenge or field virus shedding from the respiratory tract.

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